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Fluoroquinolones Inhibit, while β-Lactams Fail Against Biofilm-Producing Bacteria Isolated from Paediatric Syrups

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Background: Paediatric syrups are sugar-rich solutions widely preferred in paediatric healthcare due to their palatability, although they are highly prone to microbial contamination. Of particular concern is the presence of biofilm-producing microorganisms; existing studies have focused on contamination while overlooking the enhanced resistance mechanisms conferred by biofilm formation. This study aimed to determine the antibiogram profile of bacterial isolates from commonly prescribed paediatric syrups administered by caregivers to patients at selected healthcare centers.

Materials and methods: A total of 392 syrup sample swabs were collected from hospitals and community sources. Bacterial isolation and identification were performed using standard microbiological methods. Biofilm production was evaluated using the test-tube method, and antibiotic susceptibility was determined via the disk diffusion method.

Results: Bacterial counts ranged from $3.0 \pm 2.0 \times 10^3$ to $10.7 \pm 3.05 \times 10^3$ CFU/mL, with community samples showing the highest counts. Bacterial isolates identified included *Proteus vulgaris*, with the highest frequency of occurrence (18.75%) > *Streptococcus agalactiae*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* (12.59%) > *Pseudomonas aeruginosa*, *Arthrobacter agilis*, *Enterococcus faecium*, *Enterobacter cloacae*, *Escherichia coli* (6.25%). All isolates produced biofilms significantly different ($p<0.05$) from the negative control (broth tube without bacterial cells), except Arthrobacter agilis. Antibiotic susceptibility testing indicated multidrug resistance, particularly against amoxicillin and amoxicillin-clavulanate, while showing comparatively higher susceptibility to fluoroquinolones.

Conclusion: *P. vulgaris* was the most frequent isolate, while *K. pneumoniae* and *A. baumannii* produced the strongest biofilms. The highest resistance was observed against amoxicillin and amoxicillin-clavulanic acid, whereas fluoroquinolones remained the most effective. Paediatric syrups can harbor biofilm-producing multidrug-resistant bacteria, underscoring the importance of monitoring and safe handling.

Keywords: *paediatric syrup, antibiogram profile, biofilm, bacterial isolates, test-tube method*

Introduction

Microorganisms can exist either in free-floating, planktonic states or as complex communities of similar or distinct species known as biofilms. A biofilm is a stable community of microorganisms surrounded by a self-produced matrix composed of extracellular polymeric substances (EPS), which enables the organisms to adhere to each other and

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attach to surfaces.[1-3] Chronic, hard-to-treat, and recurrent infections caused by antibiotic resistance can often be attributed to biofilm formation by microorganisms, as well as to the emergence of slowly dividing cells known as persister cells.[4] The formation of biofilms by bacteria interferes with antibiotic therapy because the protective EPS layer prevents antibiotics from penetrating effectively and reduces stimulation of the immune system, thereby increasing bacterial survival.[5,6] The rising threat of antibiotic resistance among microorganisms is becoming a global health concern, especially in paediatric populations whose immune systems are not fully mature to fight off infections.[7] Within biofilms, there is reduced growth and metabolic activity, limited antibiotic penetration, development of persister cells, restricted nutrient availability, and an adaptive stress response. These factors collectively contribute to the high resistance of biofilms to antibiotic treatment and enable them to evade the host immune system.[8]

Syrups are concentrated sugar solutions widely preferred in paediatric healthcare because of their palatability, although they are highly susceptible to microbial contamination. A significant risk arises from the potential presence of biofilm-forming pathogens that compromise the stability and sterility of syrups. Syrups refer broadly to any oral liquid dosage form delivered in a sweet vehicle. Paediatric syrups are classified as non-sterile pharmaceutical products, meaning that even under rigorous manufacturing standards, their high sugar concentrations and organic compounds create a favourable environment for microbial growth.[9,10]

Frequent exposure of paediatric patients to contaminated oral suspensions poses a continuous risk of acquiring resistant bacteria, as children regularly take these medications. Any residual bacteria in the oral cavity can persist, leading to prolonged exposure to antibiotic-resistant strains. Once ingested, these bacteria may colonize the gastrointestinal tract and transfer resistance genes to both harmless gut flora and potential pathogens. Infections caused by multidrug-resistant bacteria can result in treatment failures, extended hospital stays, and increased morbidity.[11,12] Although these preparations are non-sterile, they are produced through rigorous aseptic techniques and undergo thorough quality control, validated by clinical trials.[13] However, at the point of administration, caregivers though well-meaning sometimes handle medications in ways that increase the risk of microbial contamination. Of particular concern is contamination by biofilm-producing microorganisms, which may exhibit increased antibiotic resistance and contribute to persistent and recurrent infections.

Furthermore, orally administered syrups containing such contaminants may introduce bacteria into the gut, potentially causing long-term consequences on the microbiome and immune function. This can result in complex health challenges, treatment failures, and diminished effectiveness of future antibiotic therapies. The challenges associated with contamination are numerous, and this research aims to assess the contamination problem arising from caregivers handling of previously opened and administered paediatric syrups.[14] This study compares microbial contamination of paediatric multidose syrups handled by caregivers in both hospital and community settings. It integrates conventional culture techniques with molecular characterization for a more comprehensive identification of bacterial contaminants. The study also highlights the specific role of biofilm in enhancing multidrug resistance (MDR) among paediatric isolates and provides insight into clinically relevant resistance trends in paediatric medicine formulations. Although previous studies have reported microbial contamination in paediatric syrups, few have combined biofilm quantification, antibiogram profiling, and phylogenetic analysis. Such integrated data remain scarce in Nigeria, leaving important gaps in understanding the genetic relatedness and resistance potential of bacterial contaminants. Addressing this gap, the present study combines molecular identification, phylogenetic analysis, biofilm quantification, and antibiogram profiling to provide new insights into the public health risks associated with contaminated paediatric syrups.[14] Therefore, this study aimed to determine the antibiogram profile of biofilm-producing bacterial isolates from previously administered paediatric syrups in selected healthcare centres.

Material and methods

Study Design and Sample Collection

A total of 392 paediatric multidose syrup samples, previously opened and administered for a at least 24 hours prior to collection, were obtained using stratified random sampling based on a modified procedure.[15] Three collection sites were included: Site A, Afe Babalola University Ado-Ekiti (ABUAD) Multisystem Hospital (AMSH); Site B, Ekiti State University Teaching Hospital (ESUTH); and Site C, community caregivers, with medications purchased from dispensing outlets and healthcare facilities. In all cases, a corresponding freshly seal-broken and immediately opened syrup sample served as the negative control. Sample size determination was calculated using the formula described in references.[16] Sterile swab sticks (Skytech Medical, Jiangsu, China) were used to collect specimens by gently swabbing the inner rim of each opened syrup bottle. The swab was immediately returned to its sterile case and transported to the laboratory for processing.[17] All samples were collected in triplicate.

Isolation and Enumeration of Bacteria

Each swab specimen was diluted in 1 mL of sterile normal saline, vortex mixed (Laboid International, Kasauli, India), and serially diluted to 10^{-2}. From this dilution, 0.1 mL was plated on sterile Nutrient Agar (Oxoid Ltd., Basingstoke, UK) and other selective/differential media using the spread plate method. Plates were incubated at 37°C for 24 hours. Colony-forming units per mL (CFU/mL) were recorded based on emerging colonies.[18]

Phenotypic Identification of Bacterial Isolates

Isolates were identified based on colony morphology, Gram staining, and standard biochemical tests, including indole, catalase, motility, methyl red, Voges-Proskauer, citrate utilization, and sugar fermentation, as described by standard bacteriological procedures.[19]

Biofilm Formation Assay

Biofilm-forming ability was assessed using the test tube method. Briefly, bacterial suspensions were inoculated into 5 mL of tryptic soy broth (TSB) (Microxpress, Verna, India) supplemented with 1% glucose (Aldon Corporation, New York, USA) and incubated at 37°C for 24 hours. After incubation, non-adherent cells were removed by washing with sterile distilled water, and tubes were stained with 0.1% crystal violet (Ugolab Productions, Kano State, Nigeria). Excess stain was washed off, and biofilm formation was visually assessed. The intensity of biofilm production was further quantified by measuring absorbance at 570 nm using a UV-Visible spectrophotometer (Scitek Global Co. Ltd, Shandong, China). Higher optical density values were interpreted as indicative of stronger biofilm production.[20]

Antibiotic Susceptibility Testing

Antimicrobial susceptibility of all the isolates was determined using the modified Kirby-Bauer disk diffusion method,[21] following Clinical and Laboratory Standards Institute (CLSI) guidelines. Bacterial inocula were adjusted to 0.5 McFarland standard and spread onto Mueller-Hinton Agar (Oxoid Ltd., Basingstoke, UK). After 5 minutes, commercial antibiotic discs were applied. The Gram-positive panel (#CT01486B; CelTech Diagnostic, Brussels, Belgium) were Pefloxacin, Gentamicin, Ampiclox, Zinnacef, Amoxicillin, Rocephin, Ciprofloxacin, Azithromycin, Levofloxacin, and Erythromycin. The Gram-negative panel (#CT01475B; CelTech Diagnostic) were Cefotaxime, Amoxicillin/clavulanic acid, Ofloxacin, Septrin, Levofloxacin, Ciprofloxacin, Amoxicillin, Gentamicin, Pefloxacin, and Azithromycin. Plates were incubated at 37°C for 18 hours. Zones of inhibition were measured in millimeters and interpreted based on CLSI breakpoints.[22-24]

Molecular Identification of Bacterial Isolates

Genomic DNA was extracted using the Zymo Bacterial DNA Miniprep Kit (Zymo Research, California, USA) following the manufacturer's instructions.[25] The bacterial 16S rRNA gene was amplified using polymerase chain reaction (PCR) in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystems Inc., Waltham, MA, USA). The PCR mixture (50 µL total volume) contained 10 µL of 5x GoTaq Colorless Reaction Buffer, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM dNTP mix, 1 µL each of 10 pmol forward primer 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 806R (5'-GGA CTA CCA GGG TAT CTA AT-3'), 0.3 units of Taq DNA polymerase (Promega, Madison, WI, USA), 8 µL of DNA template, and sterile distilled water added to bring the final volume to 50 µL.

The PCR cycling conditions were as follows: initial denaturation at 94°C for 5 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 60 seconds, and extension at 72°C for 1 minute 30 seconds; followed by a final extension at 72°C for 10 minutes. After amplification, the reaction tubes were held at 4°C.

Amplified PCR products were sequenced and analyzed using the basic local alignment search tool (BLAST) against the National Center for Biotechnology Information (NCBI) GenBank database. Phylogenetic relationships among the isolates were inferred from sequence alignments

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using Molecular Evolutionary Genetics Analysis (MEGA) software, which facilitated the construction of a phylogenetic tree using the Neighbor-Joining method to illustrate evolutionary relationships.

Data analysis

All data were analyzed using Microsoft Excel and SPSS (IBM version 30, 2024).[26,27] Descriptive statistics, expressed as mean±standard deviation, were used to summarize colony counts, biofilm absorbance, and antibiotic susceptibility results. Inferential statistics, including one-way analysis of variance (ANOVA) followed by multiple comparison post hoc tests, were performed to assess significant differences between groups. A *p-value* of less than 0.05 was considered statistically significant.

Results

Bacterial Count Exceeded Permissible Limits

Bacterial counts in the sampled syrups were higher than the permissible limit of 1×10^2 CFU/mL. Counts ranged from 3.0×10^3 to 10.7×10^3 CFU/mL across the study sites (Table 1). Community-handled samples showed the highest contamination levels (7.0±3.00 to $10.7\pm3.05\times10^3$ CFU/mL), significantly greater than those from hospital settings ($p< 0.05$).

Identification Revealed Diverse Bacterial Isolates

Phenotypic and biochemical characterization, confirmed by 16S rRNA sequencing, identified nine bacterial species: *P. vulgaris*, *K. pneumoniae*, *E. cloacae*, *A. agilis*, *E. coli*, *A. baumannii*, *S. agalactiae*, *E. faecium*, and *P. aeruginosa* (Table 2, Figure 1, Figure 2, Figure 3).

Table 1. Bacterial counts in paediatric syrup samples from hospital and community settings.

Sample Days	Study Sites (mean±SD x 10^3 CFU/mL)		
	A (AMSH)	B (ESUTH)	C (Community)
1	4.0±2.00	4.7±3.51	9.0±2.64
2	3.0±2.00	4.3±0.57	10.7±3.05
3	4.0±2.64	4.0±3.60	7.0±3.00

AMSH: Afe Babalola University Ado-Ekiti (ABUAD) Multisystem Hospital; ESUTH: Ekiti State University Teaching Hospital; Community: caregivers, with medications obtained from dispensing outlets and healthcare facilities.

Occurrence Increased for Proteus Species

The frequency of occurrence varied among isolates (Figure 4). *P. vulgaris* had the highest prevalence (18.75%), followed by *S. agalactiae*, *K. pneumoniae*, and *A. baumannii* (12.5% each). Lower occurrence rates (6.25%) were observed for *P. aeruginosa*, *A. agilis*, *E. faecium*, *E. cloacae*, and *E. coli*.

Biofilm Formation Induced Multidrug Resistance Potential

All isolates, except *A. agilis*, produced detectable biofilms (Table 3). Strong biofilm producers included *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *S. agalactiae*, *E. faecium*, *P. vulgaris*, and *E. coli*, with optical density values ranging from 1.122 to 2.956 nm. *E. cloacae* formed moderate biofilms (0.718 nm), while *A. agilis* produced weak biofilms (0.463 nm).

Antibiotic Susceptibility Profile Revealed Fluoroquinolone Activity

Antibiotic susceptibility testing showed that fluoroquinolones (ciprofloxacin, levofloxacin, and ofloxacin) inhibited most isolates effectively, with inhibition zone diameters

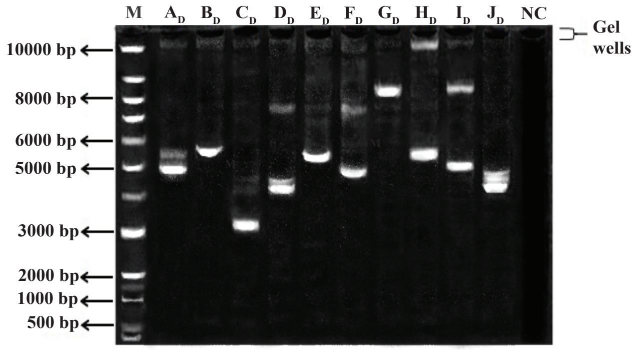

Figure 1. Agarose gel electrophoresis of 16S rRNA PCR products of bacterial isolates. Key: M = DNA size marker (1000-10,000 bp), NC = negative control. Isolates: AD = *P. vulgaris* (MW357649), BD = *K. pneumoniae* (CPO88251), CD = *E. cloacae* (KP305912), DD = *A. agilis* (OL471351), ED = *E. coli* (CPO9072), FD = *A. baumannii* (CPO001920), GD = *S. agalactiae* (NCO04368), HD = *E. faecium* (CPO16621), ID = *P. aeruginosa* (MN368594), JD= *B. subtilis* (MT998282).

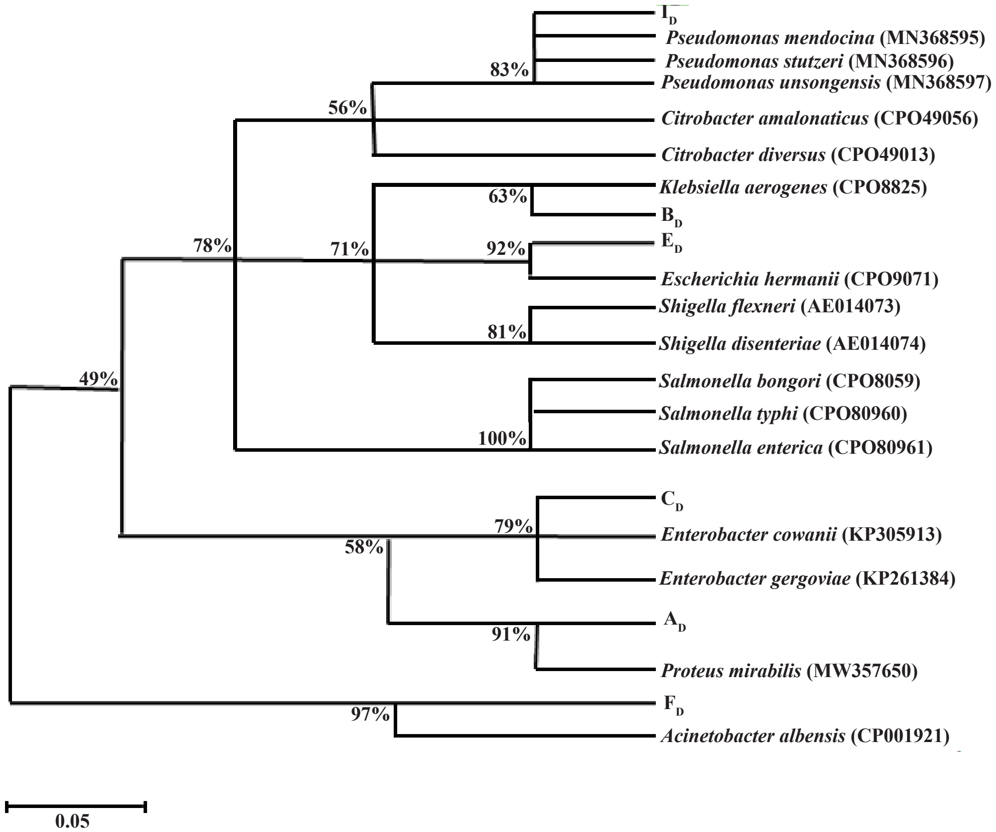

Figure 2. Phylogenetic tree of Gram-negative bacterial isolates based on 16S rRNA gene sequences. AD = *P. vulgaris*, BD = *K. pneumoniae*, CD = *E. cloacae*, ED = *E. coli*, FD = *A. baumannii*, ID = *P. aeruginosa*.

up to 27±0.35 mm (Table 4). In contrast, amoxicillin and amoxicillin-clavulanic acid exhibited the highest resistance rates, particularly against *E. coli* and *A. baumannii*. Gentamicin and azithromycin had variable effects, while cotrimoxazole (Septrin) generally demonstrated intermediate activity (Table 5).

Discussion

The results of this study demonstrated that antibiotic suspensions obtained from selected healthcare centers in Ado Ekiti, Ekiti State, were contaminated with bacteria. Oral aqueous dosage forms such as solutions, suspensions, emulsions, syrups, and tablets are particularly susceptible to

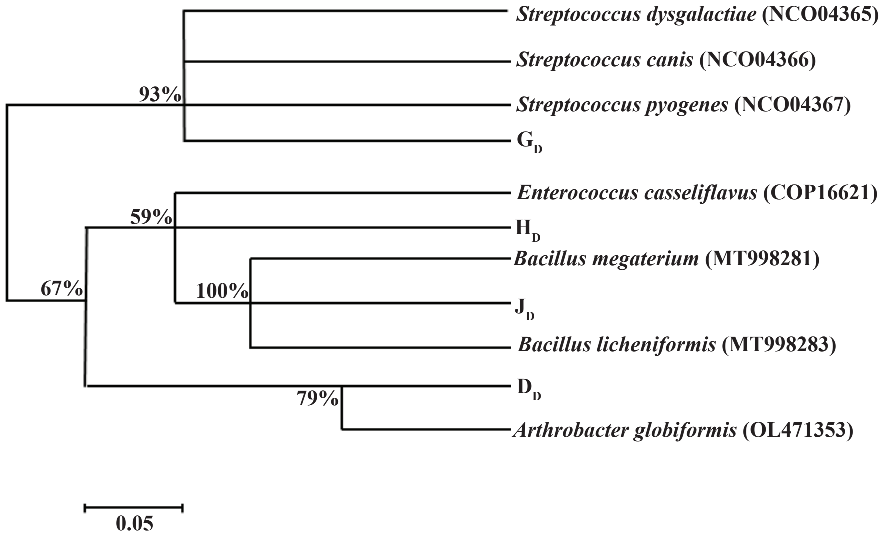

Figure 3. Phylogenetic tree of Gram-positive and actinobacterial isolates based on 16S rRNA gene sequences. DD = *A. agilis*, GD = *S. agalactiae*, HD = *E. faecium*, JD = *B. subtilis*.

Table 2. Morphological and biochemical characterization of bacterial isolates from paediatric syrups.

Isolate Code	Colonial and Cell Morphology	Gram Reaction	Catalase	Indole	MR	VP	Motility	Citrate	Glucose	Sucrose	Maltose	SSA	Mac-Conky	Probable Identity
AD	Large, milky colonies, straight rods in single or pairs	-	+	-	+	+	+	+	+	+	-	+	+	*Proteus* sp.
BD	Large, mucoid, milky colonies, entire edge, slightly curved pleomorphic rods	+	+	-	-	+	-	-	+	+	+	+	+	*Klebsiella* sp.
CD	Raised, smooth, entire edge, large mucoid colonies, rods in pairs or chains	-	+	-	-	+	+	+	+	+	-	-	+	*Enterobacter* sp.
DD	Opaque, raised, irregular colonies, rods are slightly curved in singles or short	+	+	-	+	+	-	-	+	+	-	-	-	*Arthrobacter* sp.
ED	Raised, smooth and entire edges mucoid/pinkish colonies, straight rods in chains or	-	+	-	+	-	+	-	+	-	+	-	+	*E. coli*
FD	Colonies are translucent, opaque, smooth, entire edges, rods in pairs or chains.	-	+	-	-	-	-	+	+	-	-	+	+	*Acinetobacter* sp.
ID	Large, flat greenish colonies, smooth, entire edges, rods are slightly curved and occur in	-	+	-	-	+	+	+	+	+	-	+	+	*Psedomonnas* sp.
GD	Milky colonies, cocci in pairs	+	-	-	-	-	-	-	+	+	+	-	-	*Streptococcus agalactiae*
HD	Raised, smooth, entire edge, cocci in pairs or short chains	+	-	-	+	-	-	-	+	+	+	-	+	*Enterococcus* sp.

Key: (+) positive, (-) negative.

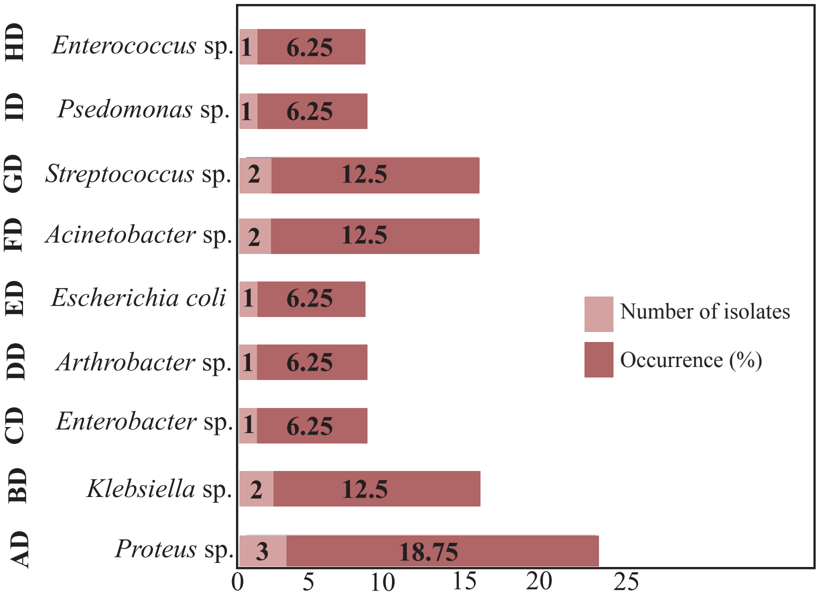

Figure 4. Percentage occurrence of bacterial isolates identified from paediatric syrup samples.

microbial contamination.[28] The microbial counts observed in this study exceeded the acceptable limit of 10^2 CFU/mL set by the British Pharmacopoeia (BP) and United States Pharmacopoeia (USP), with total aerobic microbial counts (TAMC) ranging from 3.0×10^3 to 10.7×10^3 CFU/mL. Syrups from Sites A and B showed moderate contamination, while samples from Site C were heavily contaminated. This elevated microbial load may be attributed to the high water and sugar content of syrups, which provide a conducive environment for microbial growth.[29] Additionally, poor manufacturing practices, improper storage, and repeated opening and closing of multi-dose containers can introduce contaminants from the environment or the user.[30]

Although all sites exceeded permissible colony counts, samples from Site A (AMSH/ABUAD Multi-System Hospital) ranged from 3.0 ± 2.00 to $4.0\pm2.64\times10^3$ CFU/mL, and Site B (ESUTH) showed slightly higher counts of 4.0 ± 3.60 to $4.7\pm3.51\times10^3$ CFU/mL, with no significant difference between these two sites. However, Site C (Community health caregivers) had significantly higher counts, between 7.0 ± 3.00 and $10.7\pm3.05\times10^3$ CFU/mL ($p< 0.05$), indicating poorer handling and hygiene practices in community settings compared to structured hospital environments.[31] These findings underscore the importance of proper handling and storage to minimize microbial contamination.

The results of cultural, morphological, and biochemical characterization were consistent with molecular identification. Molecular methods clarified ambiguities in cultural identification and confirmed the bacterial species, with one additional isolate, Bacillus subtilis, which was likely introduced as a contaminant during molecular processing. Gel electrophoresis profiles showed successful amplification in all test samples, with no bands in negative controls, confirming assay specificity.

The identified isolates included *P. vulgaris* (MW357649), *K. pneumoniae* (CPO88251), *E. cloacae* (KP305912), *A. agilis* (OL471351), *E. coli* (CPO9072), *A. baumannii* (CPO001920), *S. agalactiae* (NCO04368), *E. faecium* (CPO16621), *P. aeruginosa* (MN368594), and *B. subtilis* (MT998282). Phylogenetic analysis using the Neighbor-Joining (NJ) method revealed clustering consistent with bacterial taxonomy: most Proteobacteria clustered together, while Firmicutes and Actinobacteria formed separate clades, supporting correct species-level identification and evolutionary relationships. Specifically, the Proteobacteria group, including *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *E. cloacae*, *P. vulgaris* and *A. baumannii*-clustered together, reflecting their shared evolutionary ancestry and genomic relatedness. Proteobacteria are characterized by a high degree of genetic plasticity and metabolic diversity, which has contributed to their successful adaptation to diverse environments, including pathogenic niches. The grouping of these clinically important Gram-negative bacteria within a single clade confirms the reliability of molecular phylogenetics

Table 3. Biofilm formation and quantification of bacterial isolates (OD at 570 nm).

Sample Days	Biofilm Ring/Layer	OD (570 nm)
Control	-	0.251^{a}
Proteus vulgaris	+++	1.915^{b}
Klebsiella pneumoniae	+++	2.241^{bc}
Enterobacter cloacae	++	0.718^{ab}
Arthrobacter agilis	+-	0.463^{a}
Escherichia coli	+++	1.122^{b}
Acinetobacter baumannii	+++	2.956^{c}
Streptococcus agalactiae	+++	2.191^{bc}
Enterococcus faecium	+++	2.356^{bc}
Psedomonnas aeruginosa	+++	2.431^{c}

(–) absent; (±) weak positive; (++) moderate positive; (+++) strong positive. Control: broth culture tube not inoculated with bacterial vials. Significance at $p<0.05$ with respect to the negative control. Means of biofilm readings bearing different letters (a–c) are significantly different at $p<0.05$ using Tukey's multiple comparison test.

in distinguishing closely related taxa and supports their taxonomic classification within the same phylum.[32,33] In contrast, Firmicutes and Actinobacteria including *Arthrobacter agilis*, *Streptococcus agalactiae*, *Enterococcus faecium* and *Bacillus subtilis* formed separate clades, consistent with their Gram-positive cell wall structures and evolutionary divergence from Proteobacteria.Firmicutes are typically low-GC-content bacteria, while Actinobacteria are high-GC-content organisms, and these genomic distinctions are strongly reflected in their phylogenetic clustering. The distinct clades observed in the NJ tree demonstrate how molecular sequence analysis, particularly 16S rRNA-based phylogenetics, can capture evolutionary distances and correctly resolve taxonomic relationships at both the genus and species levels.

Another implication of the congruence between NJ clustering and established bacterial taxonomy is the validation of the molecular approach, confirming the accuracy of isolate identification beyond morphological or biochemical methods alone. This is particularly important in clinical microbiology, where misidentification of pathogens such as *K. pneumoniae* or *A. baumannii* could lead to inappropriate therapeutic decisions. Second, the clustering highlights evolutionary relationships, showing how pathogens with similar ecological niches or resistance mechanisms may have diverged from common ancestors. This evolutionary insight can inform antimicrobial resistance surveillance and the development of targeted therapeutic strategies.[34] Beyond validating taxonomy, the phylogenetic clustering observed in this study also carries clinical and epidemiological implications. The grouping of *K. pneumoniae* and *A. baumannii* with multidrug-resistant reference strains underscores their genetic relatedness and potential for horizontal gene transfer, which may facilitate dissemination of resistance determinants. Similarly, the close clustering of *P. vulgaris* with other Enterobacterales highlights its ecological adaptability and frequent association with opportunistic infections, while the divergence of *S. agalactiae* into a separate clade illustrates evolutionary separation yet conservation of strong biofilm-forming traits. These phylogenetic patterns suggest that the contaminants isolated from paediatric syrups are not random but share ancestry with clinically relevant MDR pathogens circulating in hospital and community environments. Such evidence strengthens the importance of integrating molecular phylogenetics into antimicrobial resistance surveillance and in tracing potential sources of contamination in non-sterile pharmaceutical products.

In this study, *Proteus* species were the predominant contaminants, aligning with similar findings from Ondo State, Nigeria, where *Proteus* spp. were the most common contaminants in cough syrups sold in patent medicine shops.[35] While part of the normal gastrointestinal flora, contamination of non-sterile pharmaceuticals with *Proteus* spp. poses a risk for opportunistic infections, especially in immunocompromised individuals.

Biofilm formation by bacteria is influenced by environmental stimuli, nutrient availability, genetic regulation, stress responses, and microbial communication. Our biofilm screening revealed that *Klebsiella* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Streptococcus agalactiae*, *Enterococcus* spp., *Proteus* spp., and *E. coli* are strong biofilm producers with optical densities ranging from 1.122 to 2.956 nm. These findings concur with previous studies reporting *Klebsiella*, *Pseudomonas*, *E. coli*, and *Acinetobacter* as strong biofilm formers.[35,36] *Enterobacter cloacae* showed moderate biofilm formation (OD = 0.718 nm), while *Arthrobacter* sp. was a weak biofilm producer (OD = 0.463 nm). Biofilm formation enhances bacterial

Table 4. Antibiogram of bacterial isolates from paediatric syrups.

Microorganism	Zone of Inhibition (mean ± SD. mm)									
	PEF (10 µg)	OFX (10 µg)	AZ (12 µg)	LEV (10 µg)	CF (10 µg)	SP (10 µg)	CPX (30 µg)	AM (30 µg)	AU (10 µg)	CN (10 µg)
Proteus vulgaris	25±3.18	26±0.70	19±2.47	23±0.35	16±5.3	13±0	27±0.35	19±8.83	3±3.88	16±7.77
Klebsiella pnemoniae	23±0.35	21±0.35	21±0.35	22±1.06	17±0.35	16±2.47	24±1.41	18±4.24	3±4.24	21±0.35
Enterobacter cloacae	23±0.35	26±1.41	12±13.7	24±1.41	19±0.7	20±0	23±0.7	22±1.76	18±1.06	21±2.47
Arthrobacter agilis	23±0.35	25±1.06	20±0.7	22±1.06	17±1.0	14±1.06	23±2.12	20±4.24	19±1.76	17±0.35
Escherichia coli	24±2.12	27±0.70	23±0	22±1.06	17±3.88	17±2.82	21±4.24	14±2.47	7±9.19	23±0.35
Acinetobacter baumannii	20±0.35	25±0.70	15±0.35	28±2.12	0	0	23±1.41	0	0	0
Streptococcus agalactiae	0	-	16±1.41	21±1.06	-	-	20±1.06	0	-	22±1.41
Enterococcus faecium	5 ± 7	-	18±2.82	22±2.82	-	-	23±2.82	0	-	21±1.76
Psedomonnas aeruginosa	24±2.12	24±1.76	22±1.76	22±2.12	17±6.0	13±4.94	23±0.35	0	0	23±3.53

PEF: pefloxacin; OFX: ofloxacin; AZ: azithromycin; LEV: levofloxacin; CF: cefotaxime; SP: septrin; CPX: ciprofloxacin; AM: amoxicillin; AU: amoxicillin–clavulanic acid; CN: gentamicin.

Table 5. Interpretation of antibiotic susceptibility patterns of bacterial isolates.

Antibiotic Agents	Disc Conc (µg)	Interpretation of Results								
		P. vulgaris	*K. pneumoniae*	*E. cloacae*	*A. agilis*	*E. coli*	*A. baumannii*	*P. aeruginosa*	*S. agalactiae*	*E. faecium*
Pefloxacin	10	S	S	S	S	S	S	S	R	R
Ofloxacin	10	S	S	S	S	S	S	S	-	-
Azithromycin	12	S	S	S	S	S	I	S	S	S
Levofloxacin	10	S	S	S	S	S	S	S	S	S
Cefotaxime	10	I	S	S	S	S	R	S	-	-
Septrin	10	I	I	S	I	S	R	I	-	-
Ciprofloxacin	30	S	S	S	S	S	S	S	S	S
Amoxicillin	30	S	S	S	S	I	R	R	R	R
Amoxicillin/ clavulanic acid	10	R	R	S	S	R	R	R	-	-
Gentamicin	10	I	S	S	S	S	R	S	S	S

S: Susceptible. I: Intermediate. R: Resistant. -: Not applicable.

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survival by enabling resistance to environmental stressors such as nutrient limitation and antimicrobial exposure.

Antibiotic susceptibility testing showed that isolates were generally susceptible to fluoroquinolones (levofloxacin, ciprofloxacin, ofloxacin), gentamicin, azithromycin, cefotaxime, and perfloxacin, but resistant to amoxicillin and amoxicillin/clavulanic acid, with intermediate susceptibility to cotrimoxazole (Septrin). Notably, Acinetobacter baumannii exhibited resistance to multiple antibiotics and had the highest biofilm optical density (2.956 nm), indicating a strong biofilm-producing capability. The protective biofilm matrix likely contributes to this resistance by limiting antibiotic penetration, leading to persistent and recurrent infections.

Biofilm-producing bacteria significantly contribute to antibiotic resistance through multiple mechanisms. Their extracellular polymeric substance matrix acts as a barrier to antibiotic diffusion and immune cell attack. Within biofilms, bacteria adopt altered metabolic states such as dormancy, reducing antibiotic efficacy against actively dividing cells.[36] This environment also facilitates horizontal gene transfer, promoting the spread of resistance genes.[37] Biofilm-associated bacteria can be up to 1,000 times more resistant to antimicrobials than planktonic cells, complicating treatment of chronic and device-related infections. Host factors, including impaired immune responses, tissue necrosis, and immune suppression, further exacerbate the challenge of eradicating biofilm-associated infections.[38]

While genetic determinants and host-related factors (*e.g.*, age, underlying disease, immunosuppression) influence antibiotic resistance, biofilm production notably enhances bacterial resilience. In young children, the mucosal immune system is structurally present but functionally immature. Secretory IgA (sIgA) levels in saliva and the gut remain low for several months and only approach adult levels by age two. This limits microbial deactivation and promotes biofilm initiation on mucosal surfaces, especially from objects like dosing syringes, droppers, and spoons. Neonatal microbiome instability and higher gastric pH further reduce colonization resistance, allowing environmental and oral bacteria to survive and form biofilms. These conditions may indirectly promote contamination of "in-use" syrups via back-contamination during repeated dosing.

Other host related factors such as underlying diseases that favor biofilms include, cystic fibrosis: thick mucus and chronic inflammation hosting *P. aeruginosa* biofilms, diabetes/hyperglycemia: elevated glucose intensifies *S. aureus* virulence and promotes biofilm formation, malnutrition: widespread in LMIC pediatric settings, diminishes mucosal and systemic immunity, increasing infection susceptibility and likely reducing clearance of biofilm communities once established as well as surgical and pharmacologic immunosuppression selects for more adherent, biofilm-competent strains *in vivo*. Device dependence (central lines, tubes) further raises biofilm risk on abiotic surfaces. These observations underscore the need for therapies targeting both biofilm disruption and immune modulation.[38] However, this study was limited to antibiotic syrups from selected healthcare centers and may not represent all brands or distribution chains. Further research, including clinical follow-up of infections in children consuming these syrups, as well as *in vivo* studies on genetic resistance mechanisms (*e.g.*, plasmid acquisition), is necessary for a comprehensive understanding.

Conclusion

P. vulgaris was the most prevalent bacterial isolate from paediatric multidose syrups, while *K. pneumoniae* and *A. baumannii* exhibited the strongest biofilm-forming capacity. Antibiotic susceptibility testing showed the highest resistance against amoxicillin and amoxicillin-clavulanic acid, particularly among *E. coli* and *A. baumannii*, whereas fluoroquinolones such as ciprofloxacin, levofloxacin, and ofloxacin retained the greatest inhibitory activity. Taken together, these findings confirm that paediatric syrups can serve as reservoirs of biofilm-producing multidrug-resistant bacteria, emphasizing the urgent need for strict microbiological monitoring and adherence to appropriate handling practices to safeguard child health.

Authors' Contributions

DDY and ED were involved in concepting and planning the research, ED carried out the data acquisition/collection and also calculated the experimental data and performed the analysis, DDY drafted the manuscript and designed the figures and ED aided in interpreting the results. DDY and ED took parts in giving critical revision of the manuscript.

Conflict of Interest

Research and all essential processes involved in this manuscript were in good standing and no conflict of interest declared nor associated with this work.

Ethical Statement

This study did not involve human participants, human biological materials, personal data, or live animals. Therefore, ethical approval from an institutional review board or ethics committee was not required.

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